CONTRIBUTIONS TO OUR KNOWLEDGE OF SOIL-FERTILITY. Nos. vii. to xi.

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vii. *The Combined Action of Disinfectants and Heat upon Soils.*

In previous researches, I have shown that the action of a moderate heat upon soil, differs from that of the volatile disinfectants, so far as the subsequent growth of bacteria is concerned. According to certain authors, the results should be similar, because both bring about the same result, namely, the destruction of the phagocytic protozoa. If the result is the same, it should be immaterial, in cases where the soil has been treated both with disinfectant and heat, whether the one is applied before the other or *vice-versa*. A preliminary test, with a garden-soil, showed a considerable difference in this respect, and led to further work upon the matter.

At first, an alluvial soil was used. The heating consisted of exposing it to 60°-65° for 30 minutes, and, where necessary, it was treated with 5% chloroform overnight. The tests were moistened with sterile water to bring the moisture to 16% and the temperature of incubation was that of the room, viz., 15°, which gradually rose to 20° as the season advanced.

Experiment i.

Alluvial soil.	Bacteria in millions per gram of air-dried soil.				
	Start.	7	15	43	64 days.
Untreated	1·2	4·8	5·3	2·1	1·5
Heated only	1·1	3·0	7·3	4·6	5·1
Heated, then chloroformed ..	1·0	4·8	6·1	5·4	6·9
Chloroformed only	...	3·1	7·2	6·2	6·8
Chloroformed, then heated ...	...	3·4	6·8	6·0	7·3

As the differences were less than had been expected, a second experiment was made, in which the moisture was brought to 19% with sterile water. This was equivalent to half-saturation.

EXPERIMENT ii.

Alluvial soil.	Bacteria in millions per gram of air-dry soil.				
	5	11	18	32	61 days.
Untreated	2·9	2·5	1·6	1·7	1·8
Heated only	4·0	2·9	1·7	2·3	2·5
Heated, then chloroformed .	5·1	6·7	5·0	6·9	5·6
Chloroformed	5·6	9·7	9·8	9·4	7·6
Chloroformed, then heated ..	5·8	7·1	6·8	8·6	7·1

These experiments show that there is only a slight difference effected by the different treatments, the small difference, however, being in favour of the preliminary treatment with chloroform. As, according to my view, the differences produced by the antiseptic treatment are caused largely by the native agricere, I continued the experiments with a soil richer in this substance than the alluvial soil which had been used.

An air-dried garden-soil was heated for 45 minutes at 62°-68°, or treated overnight with 5% chloroform. In the first experiment, No. iii., the soils were moistened with a soil-suspension containing an equivalent of 1% of raw soil, while, in the second, sterile water was employed. The chloroformed soils were difficult to moisten, and accordingly were thoroughly stirred and gently pressed flat.

EXPERIMENT iii.

Garden-soil.	Bacteria in millions per gram of air-dry soil.		
	5	11	75 days.
Control	4·2	5·6	1·5
Heated only	4·2	8·4	1·8
Heated, then chloroformed ...	36	48	8·4
Chloroformed only	78	47	17·2
Chloroformed, then heated ...	80	67	16·4

EXPERIMENT iv.

Garden-soil.	Bacteria in millions per gram of air-dry soil.			
	6	12	16	37 days.
Control	3·6	...	2·4	1·1
Heated only	3·6	...	3·6	2·2
Heated, then chloroformed ...	21	18	10·6	10·6
Chloroformed only	37	32	31·5	32·2
Chloroformed, then heated ..	33	33	32·5	32·2

The experiments show decided differences between the sets according to the order of treatment. The effect of heat is small compared with that of chloroform, and in the tests receiving the double treatment, the differences between heat then chloroform, and chloroform then heat, are marked; and it is fair to conclude that the order of treatment is not immaterial, when a soil is heated and treated with a volatile disinfectant. Why this should be so, is not clear.

The great differences in the counts of the soils treated with heat alone, and chloroform alone, especially when the soils are fairly rich in organic matter, show that the volatile disinfectant makes more nutriment available for the bacteria. The study of the nutritive effects of extracts of soils before, and after chloroform-treatment, frequently shows this in a marked manner. (Posted p. 733.)

After treatment with a volatile antiseptic, the particles of soil are undoubtedly altered physically, for such soils are difficult to wet. This has been shown by Egorow, and is confirmed by the following observations upon the capillary rise of water.

CAPILLARY POWER.

	2	20	50	73	145 hours.
Normal garden-soil ..	11·3	17	21	23	29 cm.
Chloroformed garden-soil.	0·9	2·5	2·7	3	3 cm.

Once the soil is thoroughly wetted, water probably passes as freely through it as through an untreated soil. At any rate, experiments upon the comparative rate of evaporation of water, in soils, bore this out. The trouble is in getting the treated soil thoroughly moistened, for unless this is done, the evaporation, as compared with that of an untreated soil, is slower or faster, according to whether the soil is wetted from below or from above. A thoroughly wetted and chloroformed soil lost slightly more during the first three days, but afterwards kept pace with the untreated soil.

viii. *The Toxins of Soils.*

In former papers of this series, I have shown that there are, in soils, substances which act as toxins towards bacteria. Their effect is not so clearly shown in soils themselves as in extracts obtained from them. The toxic extract, obtained by digesting soils with water and filtering through porcelain, showed its activity by either reducing the numbers of a sensitive bacterium, such as *Bac. prodigiosus,* or by retarding the speed of multiplication of the ordinary soil-bacteria. The sensitive *Bac. prodigiosus* was used, because it is typical of a class of soil-bacteria; it can be readily counted when grown upon plates, it can be evenly distributed in water, and it grows well in fluid and solid media.

The growth of bacteria, in soil-extracts, depends upon at least two factors, the nutriment in the extract, and the toxins. The former acts as an accelerant, increasing the number; while the latter behaves as a depressant, either destroying or hindering growth. In all extracts, these two play their respective parts, so that the final result will depend upon the relative preponderance of the one or the other. So far as is known, the nutrients are stable, and their effect is, therefore, constant. The toxins, on the other hand, are unstable, and according to the temperature, decay with greater or less rapidly. They are destroyed by heat and by sunlight.

As further information regarding the behaviour of the toxins should be interesting, and possibly useful, it was decided to determine the effect of extracting the soil with water under varying conditions; and, if possible, of devising a method for measuring the toxicity of soils.

The method of extracting the toxins consists in taking a quantity of the soil, and shaking it with water, 50 times every five minutes for an hour. With a very toxic soil, this is unnecessary, but with soils in which the toxicity is masked by the nutrients, the full shaking is required.

Experiment i.

	10 bacteria became	
	Stored soil.	Raw soil.
Shaken 600 times in one hour, then filtered .	125	13
Shaken 20 times in an hour, then filtered ..	290	...
Shaken 10 times, and filtered at once	...	55

The extract is then filtered through paper on the filter-pump, the first turbid runnings being returned, and the clear filtrate is filtered through a Pasteur-Chamberland F. candle. The first 20-30 c.c., are thrown away. Ten c.c. of the filtrate are pipetted into a Freudenreich flask, and seeded with 1 c.c. of a suspension of *Bac. prodigiosus,* containing a suitable number of cells.* The Freudenreich flasks are incubated overnight, and counts made by the plate-method; one-fortieth c.c. of several dilutions are smeared on set agar-plates, dried at 37°, and incubated at 28°.

In the following experiment, an air-dried garden-soil, which had been stored in the laboratory for three weeks, was extracted with distilled water.

Experiment ii.

	100 grams of dry garden-soil shaken with distilled water.	1,000 bacteria after 20 hours at 28° became
1.	75 c.c.	420
2.	100 c.c.	0
3.	150 c.c.	123
4.	200 c.c.	202
5.	250 c.c.	5,580
6.	Water-control	14

* A heaped 2 mm. loop of a 20 hours' agar-culture distributed in 10 c.c. of water by blowing, is centrifugalised until the clumps are sedimented; one c.c. of the supernatant suspension is shaken with 100 c.c. of water, and one to two c.c. of this are shaken with 100 c.c. One c.c. of this last dilution, when added to 10 c.c., and one-fortieth c.c. taken, gives a count of about 200 cells.

The graph of these numbers is interesting, but the water-control showed that distilled water had a destructive action upon the added bacteria; and as this was not desired, tap-water was subsequently employed.

In the next experiment, the soil had been air-dried for two days.

EXPERIMENT iii.

	100 grams of dry garden-soil with tap-water.	1,000 bacteria became
1.	75 c.c.	3
2.	100 c.c.	115
3.	150 c.c.	12
4.	200 c.c.	12
5.	250 c.c.	168
6.	Water-control	1,093

It is clear that the soil is toxic in all proportions used. In No. 2, the plates showed that the culture was impure, and this suggested that possibly extraneous bacteria might have an accelerating influence upon *Bac. prodigiosus.* This was tested in the following experiment, which was made nine days later, the soil having been stored for 11 days. In the second part, a drop of the unfiltered extract of No. 1 was added to each test.

EXPERIMENT iv.

100 grams air-dried garden soil, stored 11 days, with water.	1,000 bacteria in 20 hours, at 28°, became	
		in the presence of mixed soil-bacteria
1. 80 c.c.	125	7
2. 100 c.c. ...	70	33
3. 150 c.c.	1,900	1,600
4. 200 c.c. ...	16,200	16,500
5. Water-control ...	7,200	...

The soil furnished toxic extracts, although when compared with Experiment iii., the toxicity appears to be diminishing. The presence of the mixed soil-bacteria did not materially influence the growth of *Bac. prodigiosus.* Five days later, a further test of the toxicity was made.

EXPERIMENT V.

100 grams air-dried garden-soil stored 16 days, with water.	1,000 bacteria, in 20 hours, at 28°, became	
		extract diluted one-half with water.
1. 40 c.c.	200,000	...
2. 50 c.c.	1,400,000	1,200,000
3. 80 c.c.	140	380
4. 100 c.c.	12	136
5. 150 c.c.	250	980
6. 200 c.c.	230,000	...
7. 800 c.c.	180,000	...
8. Water-control ...	15,600	...

Later experiments, with this specimen of air-dried soil, showed that, upon the twenty-ninth day of storage, the toxicity had diminished considerably.

The effect of dilution upon a toxic soil-extract was studied, in order to find out, if possible, the point of dilution at which the added bacteria maintained their numbers, or, at any rate, equalled the control or water-increase. Previous experiments had shown that dilution so weakened the activity of toxin, that the nutrients caused an increase. If a balanced action could be demonstrated, a road might be found to estimate the toxicity of soils. The first experiment in this direction was upon the same soil used in the last experiment, but it had been stored for 29 days.

EXPERIMENT VI.

Equal parts of soil and water.	10 bacteria in 21 hours at 28° became
Undiluted	10,000
Diluted 1 : 1	16,400
Diluted 1 : 3	8,300
Diluted 1 : 7	4,000
Diluted 1 : 15	200
Water-control	100

The loss of toxicity, by this specimen of soil, is shown by the thousandfold increase in the undiluted extract; 13 days before, the toxicity had produced a hundredfold decrease.

At this time, the soil in the garden had been subjected to heavy rains, about 4 inches having fallen in four days, and it was not expected that it would be toxic. A trial bore this out.

Experiment vii.

Equal parts of raw soil and water.	10 bacteria, in 19 hours, at 28°, became
Undiluted	500
Diluted 4 : 1	750
Diluted 3 : 2	125
Diluted 2 : 3	13
Diluted 1 : 4	150
Water-control	25

The loss of toxicity of raw soils, being coincident with the occurrence of heavy rains, has been already noted.* That it returns again upon the cessation of the rain, and the prevalence of drying winds, is shown in the next experiment, the soil, for which, was taken after a three days' spell of cool, dry S.-W. winds.

Experiment viii.

Equal parts of raw soil and water.	10 bacteria, in 19 hours, at 28°, became
1. Undiluted	13
2. Diluted 1 : 1...	2,540
3. Diluted 1 : 3...	1,670
4. Undiluted	55
5. Second extraction of 1	1,460
6. Water-control	70

The return of the toxicity of this specimen of soil, taken 12 days after that of Experiment vii., is evident; and that it is toxic, is shown in test No. 4, which differed from No. 1, in having been shaken only ten times, and immediately filtered. The residual soil from No. 1 was extracted a second time with the same quantity of water, *viz.*, 200 c.c. to 200 grams, and the filtrate was nutritive (No. 5).

After a dry spell of five days, garden-soil was air-dried in the laboratory for three hours, and was found to contain 3·5 % moisture. It was treated with chloroform overnight, and the dis-

* These Proceedings, 1911, p.684.

infectant aired off in the morning. The same soil, without treatment, as well as raw soil taken upon the following morning, were also tested.

EXPERIMENT ix.

	100 bacteria became		
	Extract full strength.	half-strength.	quarter-strength.
Partly dried soil taken 4/6/13 ...	4	4,500	3,200
The same chloroformed	3,000	3,600	40,800
Raw soil taken 5/6/13	15	30	850

The toxicity of the soil is undoubted, and portions were used with varying quantities of water for extraction. The effect of chloroforming the soil is well seen.*

Having obtained a toxic soil, the extraction, with varying amounts of water, was continued.

EXPERIMENT x.

100 grams soil, seven days old, with water.	100 bacteria became
1. 40	85,000
2. 50	300
3. 66	5
4. 100	(2,030)
5. 133	30
6. 200	30,150
7. Water-control	290

Five (22°) and eight (28°) days later, the same sample of soil was used, as the employment of fresh soil was prevented by the prevalence of rain. The tests were incubated overnight at 22° and at 28°.

EXPERIMENT xi.

100 grams soil, with water.	10 bacteria became			
	at 22°		at 28°	
	a	b	a	b
1. 50	530	500	6,600	7,200
2. 75	600	690	6,300	7,900
3. 100	160	530	5,800	(12,000)
4. 150	(1,170)	70	3,100	3,900
5. 200	60	40	3,700	4,700
6. 250	230	220	2,500	2,300
7. Water-control ...	40	12	90	100

* Compare these Proceedings, 1910, 814.

In this and the preceding experiment, the bracketed numbers are probably excessive. It is frequently found, in a series of tests, that one is out of what appears to be the normal sequence. For this reason, the last experiment was made in duplicate, so that a discrepancy might be allowed for.

It is seen that the toxicity of the soil, manifest in Experiments ix., and x., has disappeared by the twelfth day of storage, but although now nutritive, there is some confirmation of the toxicity being most evident when about equal parts of soil and water are taken.

The return of the toxins to the soil, after dry weather, shows that, though they may be disguised by the soil-nutrients, they should be reckoned with, when considering the seasonal variation of bacteria in soils. The seasonal variation has been noted by many writers. Conn found that the numbers were high in February, and, rising in the summer, fell again in the autumn. Hiltner and Störmer showed that the bacteria did not tend to increase as the temperature rose, the August counts being no higher than those of February, and, in some cases, they were less. It is true that one cannot trace a direct relationship between the rainfall and the bacterial numbers of some investigations,* but the question may have to be determined in tropical or subtropical countries, where the rain falls during regular monthly or quarterly periods. The removal of toxin, by drainage-waters, is another question that deserves consideration.

From the irregularity of the results obtained by diluting the soil-extracts, it would appear that this method offers no means of determining the toxicity of soils. As the toxins are thermolabile, the action of heat might prove more successful; and, accordingly, experiments were made in this direction. Two years previously* it had been found that, in a certain soil-extract, 1,000 bacteria became reduced to 73; but when the extract had been raised to boil-

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* As, for example, Engberding, Centrl. Bakt. 2te., 23, 569.

† These Proceedings, 1911, 815.

ing point, they were reduced only to 667; and, when boiled for an hour, they increased to three and one-quarter millions.

Extracts of the garden-soil at various depths were made, in order to see to what extent the toxins had been washed down by previous rains, and instead of subjecting the extracts to 100°, the lower temperature of 60° was employed. The top inch of the soil was removed, and the succeeding three-inch portions were taken. The two lower layers contained some clay, which had probably been put there when the sandy soil had been used as a garden, about twenty years ago.

Experiment xii.

Moist garden-soil.	Moisture %.	10 bacteria became		
		Extract not heated.	Heated at 60° for	
			20 minutes.	60 minutes.
First three inches	14·3	1,500	1,130	1,110
Second three inches	10·3	1,060	810	1,060
Third three inches ...	11·1	160	65	56
Water-control	...	13	...	...

The first and second three inches, which, together, may be taken as representing the soil, have much the same nutritive power, and have much the same behaviour. The third three inches, which may be taken as the subsoil, is much less nutritive. The toxin is apparently different, for heat, at 60°, does not increase the nutritive effect. It does not appear that the soil-toxin has been retained by the subsoil, unless it is that the rain of the previous three days had been excessive. On the 28th, 29th, and 30th June, the soil received 3, 25, and 71, points of rain, respectively, that is, a total of, practically, an inch of rain upon the three days preceding that on which the soil was taken. The conditions were, therefore, against finding toxin in the soil. There appears to be an increase of toxicity upon heating the extracts of the subsoil at 60°, or, what may be the equivalent in this case, a diminution of nutritive effect, which does not occur in toxic soils. The portions of soil were cut out with a circular tin-cylinder of 3·5 inches diameter, and three inches deep. Each portion weighed approximately 400 grams. The inch of rain, which fell upon the surface-area, measured approximately 160 c.c. Each portion, therefore, held 57, 41, and 44 c.c. respectively, or 142 c.c. in all, so that the soil had been leached by the 160 c.c. of rain.

The experiment was repeated in a modified form, a week later, and during this time, no rain had fallen. A saline solution, containing 0·2 % of potassium sulphate, was used in making the extracts, as the flocculation of the clay-particles, by water, retarded the filtration of the soil-suspensions. Instead of heating the extracts at 60°, they were boiled in flasks fitted with aërial condensers. In this, and the previous experiment, 400 grams of moist soil were shaken with 400 c.c. of tap-water or saline for an hour.

Experiment xiii.

Depth of garden-soil.	Moisture.	10 bacteria became			Bacteria in 1 gram. of dry soil.
		Unboiled.	Boiled		
			15 min.	60 min.	
2 to 4 in. ...	9·7%	6	17	1,350	5,200,000
10 to 12 in. ...	10·3%	11	10	2	2,500,000
Saline-control ...	...	18	...	...	...

The soil is decidedly, though feebly, toxic, and, according to expectation, the toxins were destroyed by heat, allowing the nutrients to produce an increase of bacteria. The subsoil is also toxic, inasmuch as the unboiled extract produced fewer bacteria than the saline control. The action of heat, upon the subsoil-extract, is in contrast with the soil-extract, but is confirmatory of the previous experiment. Thus there appear to be two toxins in soil, one thermolabile, the other thermostable, unless it is that the latter is a product of the action of heat upon some soluble and filterable soil-constituent. Prolonged or excessive heat develops thermostable toxins in the soil itself, and the thermostability of the extracts of the subsoil, and, in some cases, of the soil, may be due to a similar phenomenon. As it is, we have to deal with a complex action.

Fifty days later, the soils were again tested. No rain had fallen for nearly a month, and the soil was consequently very dry.

EXPERIMENT xiv.

Depth of garden-soil.	Moisture.	10 bacteria became		
		Unboiled.	Boiled 15 min.	Boiled 60 min.
$1\frac{1}{2}$ to $4\frac{1}{2}$ inches	4·6%	125	775	2,800
9 to 12 inches	7·1%	22	7	0
Saline-control	...	37	...	...

The soil was nutritive, but, as shown by the effect of heat, it contained thermolabile toxins. The subsoil was toxic, and the toxicity was increased by boiling the extracts. One expected to find the soil strongly toxic after the spell of dry weather, and the failure to realise this, shows that an accumulation of toxic substances does not occur in dry soils. The condition is similar to that which takes place in the laboratory, with soils that are air-dried and stored. They rapidly lose their toxicity and become nutritive. Inferentially, a certain percentage of moisture is necessary for the formation of toxins in soils.

From these experiments, it is seen that the demonstration of toxins in soils depends upon obtaining a soil in which the toxins preponderate over the nutrients, and in using an appropriate dilution in making the extracts. Equal parts of soil and water generally yield the most toxic extract. The toxins of the soil are thermolabile, while those of the subsoil used were thermostable. The existence of two kinds of soil-bacteriotoxins are thus indicated.

ix. *The Formation of Toxins in the Soil.*

The leaching out of the thermolabile toxins from soil by rain, the occurrence of thermostable toxins in the subsoil, and the reappearance of thermolabile toxins in the soil, make it appear probable that thermolabile toxins are produced entirely in the soil, and do not rise from below. The production may take place rapidly, as a sample taken after a rainfall of 4·75 inches, and stored in the laboratory for three days, showed the following:—

EXPERIMENT i.

	100 bacteria at 22° became
Raw extract, unboiled	3
Extract boiled ½ hour	1
Extract boiled one hour	20
Saline-control	228

Again, a soil was extracted at once, and after storage in a bucket, in the laboratory, for seven days, during which, the moisture fell from 10% to 8%.

EXPERIMENT ii.

	100 bacteria at 22° became			
	Unboiled.	Boiled 15 min.	Boiled 60 min.	Control.
Raw soil	60	170	13,650	180
After seven days' storage ...	0	7	360	127

A soil, which was nutritive in the fresh condition, was incubated at 28° for 14 days. Unfortunately, the plug in the bottle permitted the soil to dry, and the moisture fell from 10% to 4·5%, so that the conditions for toxin-formation and preservation were not the most favourable. However, the soil became toxic, as the following shows:—

EXPERIMENT iii.

	10 bacteria became						
				Diluted.			Water-control.
	Undiluted.	4/5	3/5	2/5	1/5	1/10	
Fresh soil	187	107	124	210	73	38	18
The same incubated 14 days at 28° ...	106	4	13	970	14	41	164

The increase of toxicity is seen upon comparing the undiluted numbers with the water-controls. It is also seen in all dilutions, excepting the two-fifth. The effect of dilution is peculiar, but, beyond recording the counts, little can be said, at present, regarding the matter.

A spell of dry weather prevented the continuation of similar tests at the time, and, therefore, a quantity of soil was put into a Büchner porcelain-funnel, and treated, during one day, with a quantity of distilled water, equivalent to one inch of rain. On the following day, the drainage was filtered and tested, the soil mixed and divided into portions, each containing 200 grams of dry soil. These were stored in bottles at 22°, and tested from time to time.

Experiment iv.

	100 bacteria at 22° became			
	Unboiled.	boiled ¼ hr.	boiled 1 hr.	Control.
Soil-drainage...	270	510	3,640	100
Soil extracted at once ...	1,910	4,640	5,520	100
Soil extracted after 5 days ..	104	534	10,090	550
Soil extracted after 13 days.	380	875	23,600	600
Soil extracted after 33 days.	113	41,000	54,600	900
Soil extracted after 49 days.	56	2,400	23,000	194

A rich, brown, alluvial soil, from the Hawkesbury Agricultural College, had been stored in the laboratory for about a year. It contained 1·5% of moisture. Two hundred gram-portions were weighed out into 700 c.c. bottles, and moistened with 40 c.c. of a soil-suspension of the same soil, which had been growing maize-plants in the glasshouse. Ten grams of the soil were shaken with 500 c.c. of sterile water, to make the suspension. The bottles were corked, and divided into two sets, one being kept at 28°, the other at laboratory-temperature (15° to 20°). When required, each bottle received 160 c.c. of water containing 4 c.c of 10% potassium sulphate. The shaking and filtration were done in the usual manner.

Experiment v.

	100 bacteria became at 22°						
	Soil incubated at 15°-20°.			incubated at 28°.			Control.
	unboiled extract.	boiled 15 min.	boiled 66 min.	unboiled extract.	boiled 15 min.	boiled 60 min.	
At start ...	35,000	129,000	476,000	35,000	129,000	476,000	370
After 6 days..	9,000	34,000	78,000	1,500	12,000	43,000	390
After 30 days.	420	6,000	24,600	3,900	9,000	17,800	194

The experiment shows that a soil, from which the toxins had decayed during long storage, became less nutritive or more toxic upon being moistened. At a comparatively low temperature (15°-20°), the change was slower to develop than at a higher temperature (28°), but when formed, it was more persistent.

Observations made, during these investigations, show that soil has a variable, bacteriotoxic content. Rain washes the toxin out, and the soil becomes non-toxic. When the rain ceases, bacteriotoxins are again formed, and persist, if the soil remains moist. With continued dry weather, and consequent lowering of the soil-moisture, the toxins decay. Experiments in the laboratory confirmed these observations. A soil originally toxic, became non-toxic when washed with water, and, upon incubation, again became toxic. Another soil, originally nutritive, became very much less so upon incubation.

x. *The action of Chloroform upon Blood treated with Vaseline.*

When a soil is treated with a volatile disinfectant, it behaves as if more nourishment had been liberated for the growth of the surviving bacteria. The volatile disinfectants are also fat-solvents, and, after noting their visible action in the soil, I suggested that the effect, produced by the disinfectant, was by virtue of its removing the fat or agricere, and so facilitating the decay of the organic matter. Certain experiments* upon the growth of bacteria, in various layers of disinfected soil, bore out this suggestion. In endeavouring to confirm the hypothesis, I have made experiments with the ammoniacal fermentation of blood saturated either with paraffin or vaseline, and treated with chloroform. These, however, are rather against the hypothesis, and are here recorded as a contribution to the subject.

Dried blood was heated for a day, at 56°, with paraffin melting at 43°, and the excess removed. After being ground and sifted, two gram portions were weighed out, mixed with 50 grams of sand, and treated with chloroform. One hundred and thirty grams of dry soil were added, and 30 c.c. of soil-infusion. The tests were incubated at 22°, for 6 days; then water was added,

* These Proceedings, 1911, pp.696 *et seq.*

and the bottles shaken for a day, and the extract filtered off in the morning. The filtrates were distilled with magnesia, and the ammonia determined.

Experiment i.

	Milligrams of ammoniacal nitrogen.
Paraffined blood, untreated... .	41 43 38 —41
Paraffined blood, chloroformed ..	39 43 41 —41

Experiment ii.

	Milligrams of ammoniacal nitrogen.
Vaselined blood, untreated	60 60 65 —62
Vaselined blood, chloroformed ...	53 53 61 – 56

The next experiment was made with vaselined blood and sand. No soil was used, and, as a fermenting agent, a suspension of *Bac. prodigiosus* was added. A tube containing a strip of paper, moistened with dilute sulphuric acid, was placed in the test-bottle, to prevent any possible loss of ammonia.

Experiment iii.

	Milligrams of ammoniacal nitrogen.
Vaselined blood, untreated	13·4 12·3 12·5 13·7 ——13·0
Vaselined blood, chloroformed ...	13·4 14·1 11·9 11·5 ——12·7

The experiments show that dried blood, saturated with paraffin or vaseline, and afterwards treated with chloroform, does not decay quicker on account of the chloroform-treatment.

xi. *The Action of Naphthalene in Soil.*

The action of certain chemicals, used as fungicides, in increasing the yield of crop, has been frequently noted. Of these, perhaps, the most conspicuous example is the effect of spraying potatoes with Bordeaux mixture. As a rule, the treatment shows that the mixture has a decided manurial effect. We do not know, however, whether it is the lime or the copper that produces the result, and, in a series of tests upon the growth of bacteria in soils, that I made with copper sulphate, lime, a mixture of these and with superphosphate, the results were negative; that is to say, the control-test showed a greater number of bacteria, from time to time, than any of the others.

Meanwhile, Mr. Hugh Dixson called my attention to the proposed use of sulphur and naphthalene in horticultural practice. Both are said to augment the crop in unsterilised soils, and I decided to test them, with regard to any action they might have upon the increase of bacteria. The sulphur was used as precipitated sulphur, and the naphthalene as "vapo-naphtha," generally used, in conjunction with lime, for destroying injurious insects. In a previous paper, I showed that sodium thiosulphate increased the growth of bacteria in soil-extracts, and, as the same might occur in soils, it was used with other salts in an experiment. The test was made upon soils contained in bottles closed with a cork furnished with a glass tube drawn out to an open capillary point, and were incubated at 22°.

Experiment i.

Garden-soil, with 16% moisture.	Bacteria at 22° in millions per gram of air-dried soil.				
	6 days.	19 dys.	28 dys.	47 dys.	123 dys.
Precipitated sulphur, 0·1%	4·8	2·4	2·2	1·2	0·6
Naphthalene, 0·1%	90·0	10·7	10·5	23·4	8·0
Sodium thiosulphate, 0·1%	9·8	15·8	16·8	15·0	2·2
Control	2·4	1·6	1·0	1·2	1·1
Calcium sulphate, 0·1% ...	3·4	0·9	0·2	0·8	0·7
Ferrous sulphate, 0·05%	0·4	0·6	1·0	0·8	0·6
Copper sulphate, 0·04%	0·6	0·5	0·7	0·7	0·7

As 0·1% is approximately equivalent to 27 cwts. per acre, the quantities added were excessive, but they have shown that naphthalene and thiosulphate have a decided effect upon the numbers of bacteria. In the case of the naphthalene, the amount used was about eighty times that recommended, assuming that it was thoroughly mixed with the soil. The sulphur was about ten times that used by Boullanger in his experiments. The prevailing bacterium, in the six days' naphthalene-test, was *Bact. putidum* (85 millions).

A second experiment was prepared, using varying quantities of naphthalene and thiosulphate. The soil was an alluvial, and received raw soil-extract equivalent to 1% of soil. The bottles were stored in the laboratory.

EXPERIMENT ii.

Alluvial soil.	Bacteria in millions per gram (18°-27°).				
	6 days.	12 dys.	18 dys.	28 dys.	99 days.
Control	1·2	2·1	2·4	1·6	1·8
Sodium thiosulphate, 0·075%	1·7	2·9	2·6	2·5	2·8
Sodium thiosulphate, 0·15%	2·3	2·7	2·2	1·4	2·2
Naphthalene, 0·0187%	21·7	6·2	8·8	5·6	3·0
Naphthalene, 0·0375%	5·8	21·2	18·4	18·8	3·9
Naphthalene, 0·075%	5·6	32·1	23·0	30·2	4·7

In this soil, the thiosulphate does not show the difference over the control that it did in the previous experiment with garden-soil, while the behaviour with naphthalene is confirmed. The different action of thiosulphate, in the two kinds of soil, may possibly be explained by the greater agricere-content of the garden-soil, which is comparatively rich in this substance, while the alluvial soil is comparatively poor. Seymour Jones* says that sodium thiosulphate possesses the property of being able to remove grease from pelt or from leather, and it may have the same effect, therefore, upon the organic matter of the soil.

Naphthalene is used as an insecticide, and to disguise disagreeable odours, such as occur in urinals, etc. It is soluble in oils,

* Journ. Soc. Chem. Ind., 1912, 1130, abstract.

a solution in olive oil being used in cases of scabies. Molten naphthalene dissolves paraffin, grease-paint, unguentum resinæ, etc., and it acts, therefore, much as a volatile disinfectant. That its antiseptic value is exceedingly poor, is evident from the numbers of bacteria obtained in the experiments. This, however, applies only to the quantities taken; a larger quantity might show a disinfecting action. Small quantities of certain poisons, such as ether, carbon bisulphide, potassium bichromate, copper sulphate, etc., have a stimulating influence upon bacterial growth, while certain others have not;* and it is possible that the naphthalene, in the experiment, exerted an accelerating action.

The great multiplication of *Bact. putidum*, in the first experiment, points to the probability that naphthalene will induce an increased ammonification. To prove this, an experiment was made by adding two grms. of dried blood to 200 grms. of dried garden-soil, and adding a soil-suspension, made by shaking 100 grams of raw soil with a litre of water. With this, the moisture-content was made up to 19%. The bottles, containing the soil, stood upon the laboratory-bench for seven days, when they received two grms. of copper sulphate, and 500 c.c. of water. They were shaken 50 times, at hourly intervals, for a day, allowed to rest overnight, and the supernatant liquid filtered in the morning. The ammonia was determined, in the usual way, by distilling with magnesium oxide. The numbers are the average of three, and sometimes of four tests.

Experiment iii.

Milligrams of naphthalene added to 100 grams soil.	Ammoniacal nitrogen formed from 2 grams of dried blood in 7 days; milligrams.
None.	66
0·5	64
5	63
25	62
50	21

This unexpected lowering of the ammonification led to another experiment being made.

* Fred, Centrlbl. f. Bakt., 2te Abt., 31, 185.

EXPERIMENT iv.

Milligrams of naphthalene added to 100 grams soil.	Ammoniacal nitrogen formed from 2 grams of dried blood in 6 days; milligrams.
None.	60
1	60
10	58
50	35
250	23

It is clear, that when 1% of dried blood is contained in soil, naphthalene acts as a depressant, so far as ammonification is concerned. In the experiments with soil and naphthalene, the latter showed itself to be an accelerant of bacterial growth, and, inferentially, of the rapidity of decay. The sets of experiments are, therefore, at variance. In an endeavour to find the cause of the variance the following was obtained.

EXPERIMENT v.

Milligrams of naphthalene added to 100 grams soil.	Ammoniacal nitrogen formed from 2 grams dried blood in 4 days at 21°; milligrams.	Bacteria in millions per gram of dry soil.
None.	36	45
1	34	44
10	26	45

The result shows that, under the conditions of the experiment, there is no difference in the bacterial counts; and that, in the presence of dried blood, the stimulating action of naphthalene is not evident.

As it would be interesting to know what ammonification occurs when unmanured soil is used, a similar experiment, without the blood, was made. This extended over 14 days, and the result showed that no ammonia had been formed from the organic matter of the soil; that is to say, no ammonia was detected, upon distilling the soil-extracts with magnesium oxide. In spite of this, the bacterial counts showed a rise almost proportional to the amount of naphthalene added.

EXPERIMENT vi.

Milligrams of naphthalene added to 100 grams soil.	Bacteria in millions per gram, grown upon Lipman-Brown synthetic agar.				
	Total colonies.	True bacteria.			Cladothrix Moulds.
		Mixed types.	Translucent.	Gummy.	
None.	3·7	1·5	0·1	0	2·4
1	4·9	2·7	0·2	0·2	1·8
10	11·7	4·5	1·5	0	5·7
50	47·0	12·2	14·1	0	20·7
250	98·0	19·6	58·8	5·9	13·7

The great bulk of the translucent colonies consisted of a small, inert cocco-bacterium.

Some pot-experiments were made with oats and maize, in soil containing none, 0·001, and 0·005 per cent. of naphthalene; but although the plants started somewhat better in the naphthalened soils, the others soon overtook them, and ultimately there was no difference.

The conclusion arrived at, from these experiments, is that while naphthalene induces an increase in the number of bacteria in unmanured soils, there is no corresponding increase in the formation of ammonia from the organic matter originally present or added as dried blood.